(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 April 2004 (08.04.2004)

PCT

(10) International Publication Number
WO 2004/029294 A1

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number:
PCT/SE2003/001499

(22) International Filing Date:
26 September 2003 (26.09.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0202867-8	27 September 2002 (27.09.2002)	SE
60/413,752	27 September 2002 (27.09.2002)	US

(71) Applicant *(for all designated States except US)*: **BIOTAGE** [SE/SE]; Vallongatan 1, S-752 28 Uppsala (SE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **TOOKE, Nigel** [SE/SE]; Olof Thunmans väg 14, S-741 44 Knivsta (SE).

(74) Agent: **ALBIHNS STOCKHOLM AB**; P.O.Box 5581, Linnégatan 2, S-114 85 Stockholm (SE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/029294 A1

(54) Title: NEW SEQUENCING METHOD FOR SEQUENCING RNA MOLECULES

(57) Abstract: The present invention provides a method for determination of the identity of at least one nucleotide in a RNA-molecule comprising the steps of: (i) providing the RNA-molecule, an oligonucleotide primer binding to a predetermined position of the RNA molecule, a reverse transcriptase, deoxynucleotides and other necessary reagents, in a reaction vessel; (ii) performing a primer extension reaction, whereby the oligonucleotide primer is extended on the RNA-molecule through incorporation of at least one deoxynucleotide by the action of a reverse transcriptase, resulting in the release of a PPi molecule only upon incorporation of a deoxynucleotide; and (iii) detecting the presence or absence of incorporation, thereby indicating the nucleotide identity of the RNA molecule in the relevant position. In a preferred embodiment, the sequencing of the invention is coupled to the Pyrosequencing™ reaction. A variant of the method employs incorporation of modified nucleotides, with an optionally cleavable linker arm to which is attached a label.